FUNDAMENTAL RESEARCH AT THE [BIO:INFO:MICRO] INTERFACE

High-resolution analysis and modeling of temporal and spatial patterns of biological control circuits

Stanford University
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High-resolution analysis and modeling of temporal and spatial patterns of biological control circuits

Interdisciplinary Stanford University team

Harley McAdams (PI)	Developmental Biology
Stanley Cohen	Genetics
Stephen Smith	Mol. & Cell. Physiology
Lucy Shapiro	Developmental Biology
Matthew Scott	Developmental Biology
James Harris	Electrical Engineering
Olav Solgaard	Electrical Engineering
Martin Morf	Electrical Engineering
Claire Tomlin	Aeronautics/Astronautics
W. E. Moerner	Chemistry

High-resolution analysis and modeling of temporal and spatial patterns of biological control circuits

Overall objective

Analyze fundamental mechanisms regulating the cell cycle and cellular differentiation, including differentiation of multicellular organisms with a program of

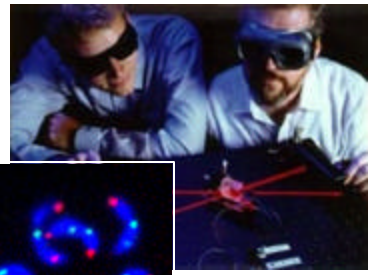
- **Enabling instrumentation technologies**
- **Informatics and biosimulation advances**
- **Biological studies**

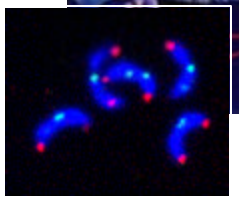
Factors Driving Biological Progress

- **Robotics, nanotechnology, and optoelectronics applied to high-throughput experimental instrumentation**

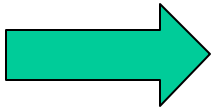
- **Bioinformatics**

- **Fluorescent tagging of bio-molecules and *in vivo* imagery**





- **Coupling of bacterial studies with studies of parallel questions in embryos to exploit evolutionary conservation of mechanisms**

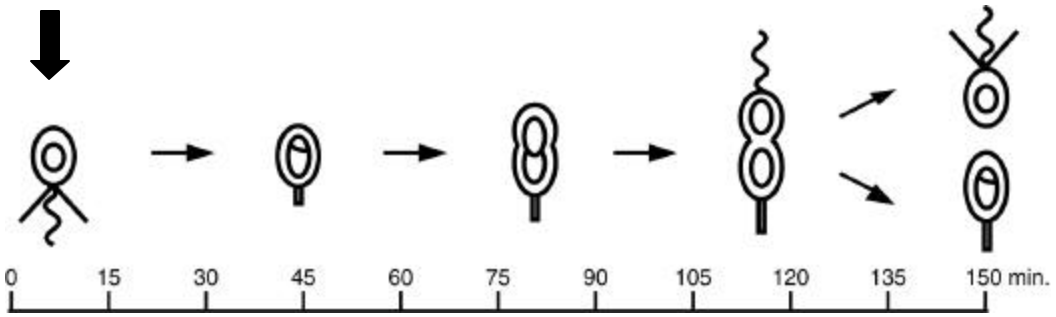


Collaborations

McAdams/Shapiro	• **Apply gene expression microarrays to deduce the complete regulatory circuit controlling cell cycle and asymmetric division in a bacterium, *Caulobacter crescentus*, with 3,400 genes**
Moerner/Shapiro	• **Apply high-resolution in vivo fluorescent microscopy to characterize mechanisms that localize regulatory proteins in *Caulobacter crescentus***
Smith/Harris	• **Develop low-cost, fluorescence reader/microscope integrated on a single-chip for field-deployable bioassay instruments**
Smith Scott/Cohen/	• **10X enhancement of 2-photon microscopy throughput and application to time-lapse imaging of development in living embryos and bacterial colonies**
Scott/Solgaard	• **Develop nanotechnology-based instrument to inject aliquots of regulatory molecules into specifically-targeted cells of 1000 *Drosophila* embryos per hour**
Cohen/McAdams	• **Apply artificial intelligence and genetic simulation technologies to deduce genetic circuitry from gene expression microarray data**
Tomlin/McAdams/Morf	• **Apply hybrid control theory and logic synthesis to analysis of control systems guiding somatic development in *Drosophila* embryos and other multicellular systems**

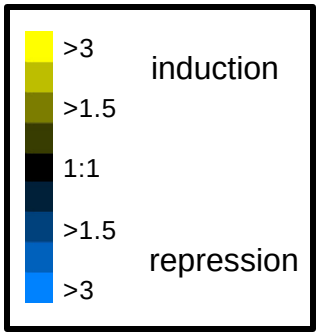
Cell Cycle Time Course

synchronize cells



- collect **RNA** every 15 min. for 150 min.
- hybridize each time point to common **reference RNA** derived from a mixed, unsynchronized culture

ftsZ

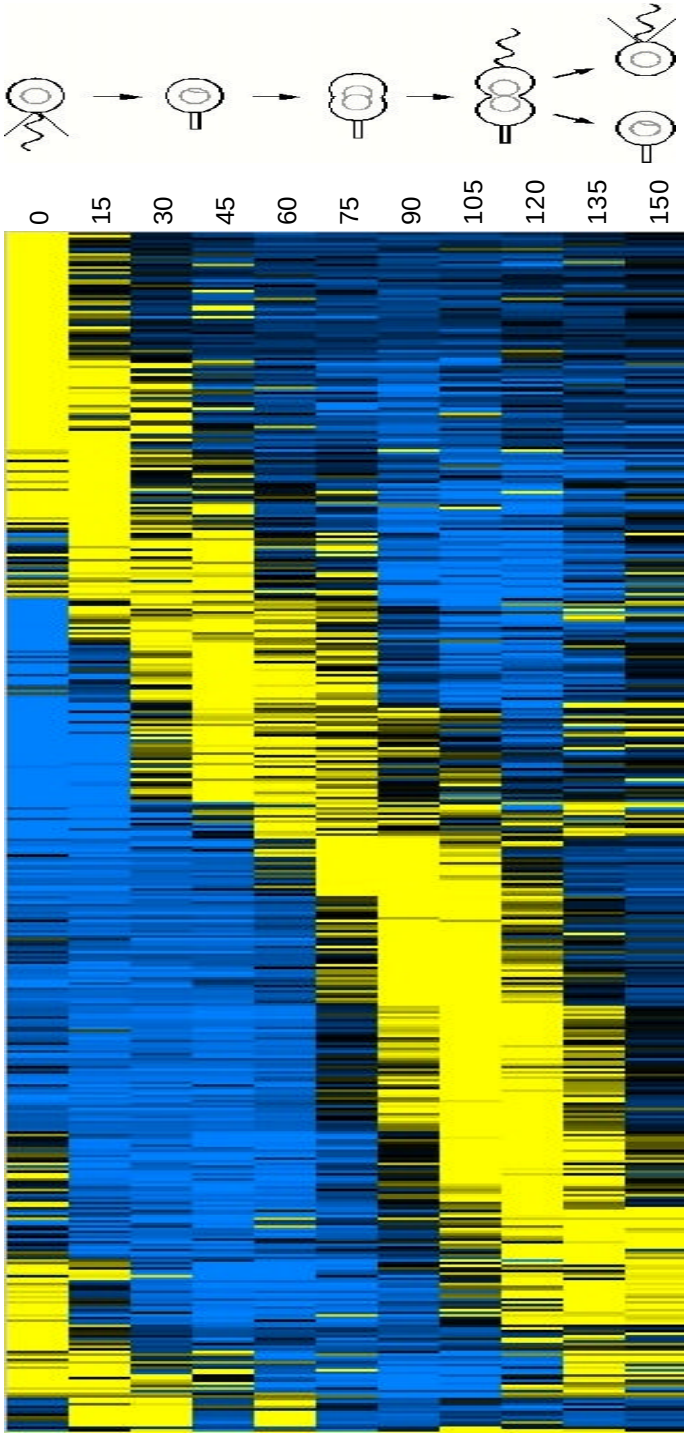


2966 expression profiles

553 cell cycle-dependent transcripts → clustering analysis

Microarrays
- **Michael Laub**

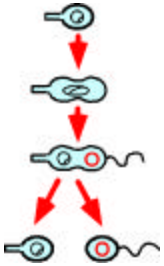
Caulobacter **genome sequence**
- **The Institute for Genome Research (TIGR)**



Experimental Tactics

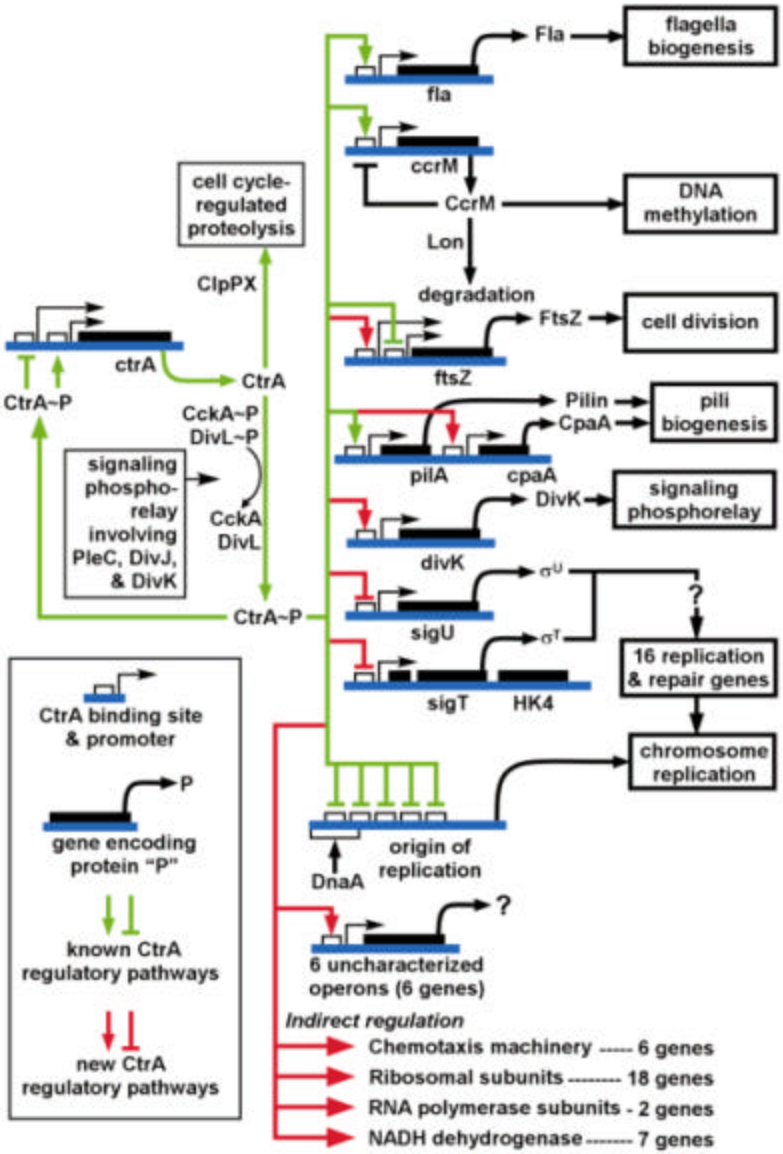
- **Expression profiles of mutants versus wild type**
- **Expression profiles of wild type under different conditions**
- **Identify common promoter motifs in temporally co-regulated genes**

Caulobacter Crescentus Development



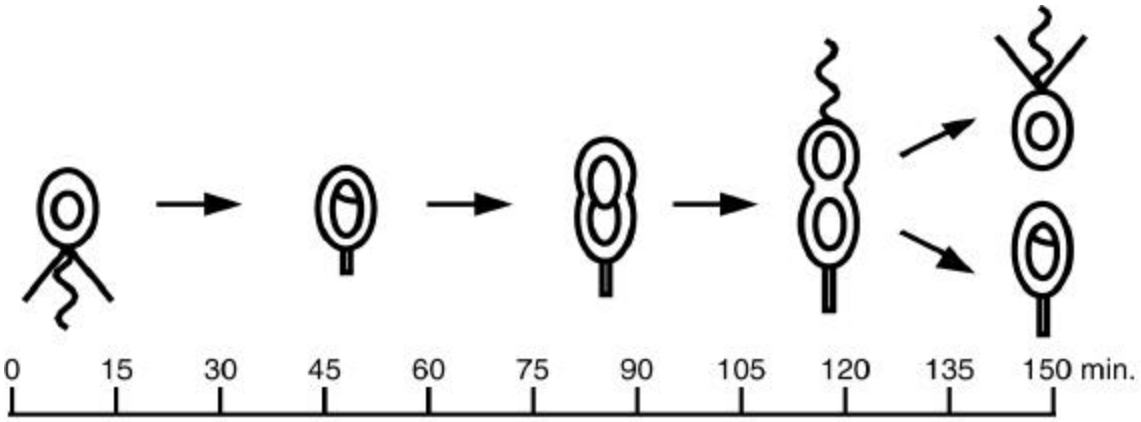
- **~553 cell cycle-controlled genes (~19%)**
  - **CtrA master regulator directly or indirectly controls >26%**
- **40 cell cycle-regulated two-component signaling proteins**

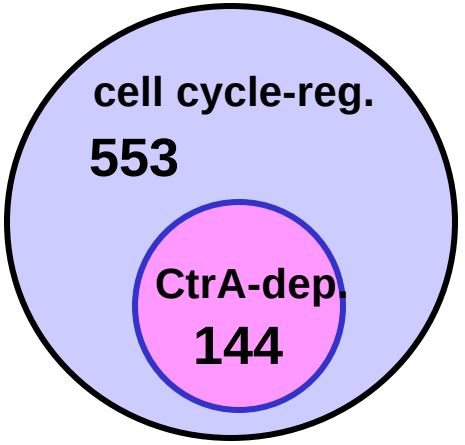
Objective of this project: identify the complete regulatory circuitry controlling a bacterial cell cycle



Laub, McAdams, Feldbluym, Shapiro. *Science* (2000) In press

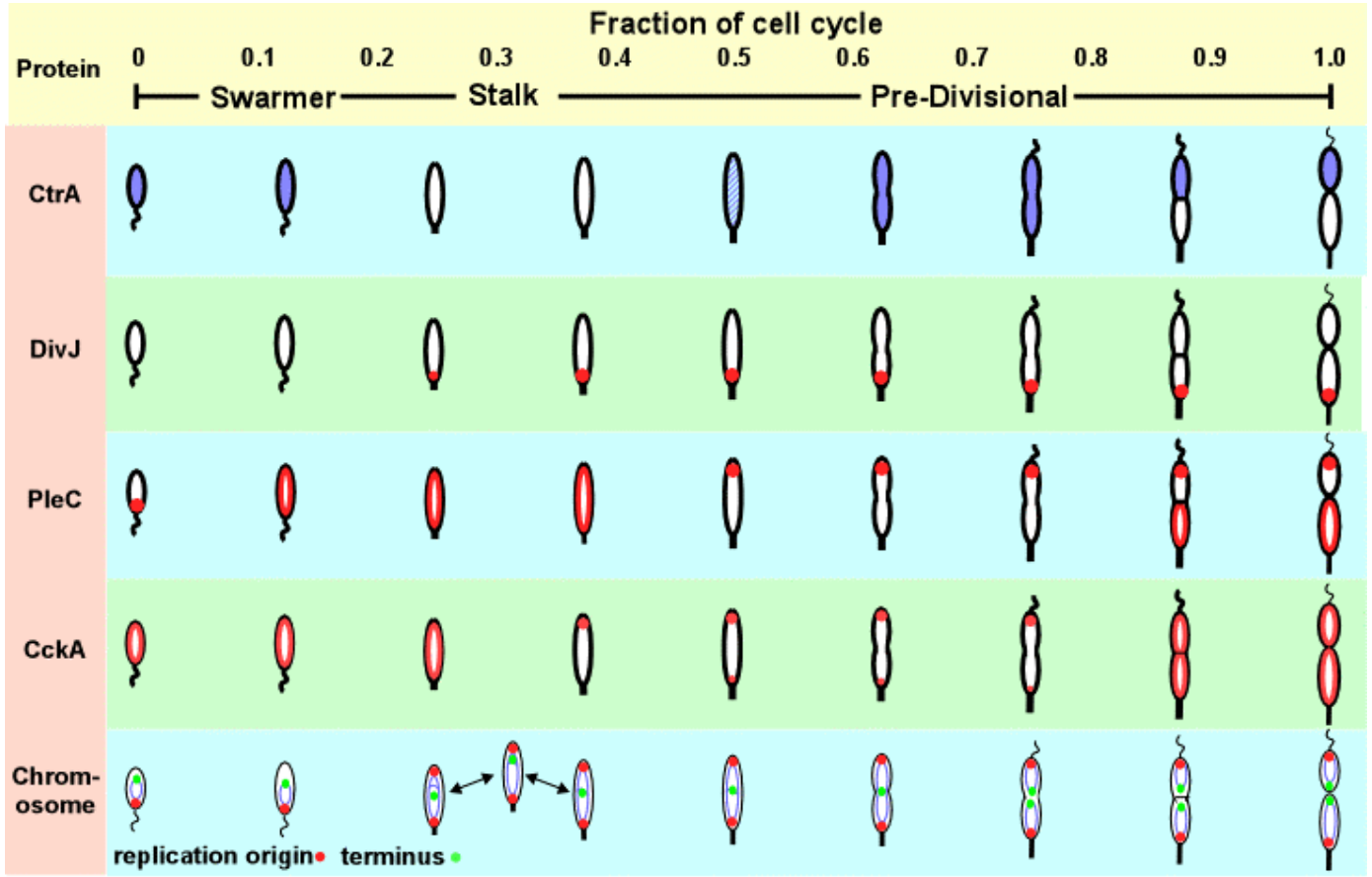
Cell Cycle-Regulated Regulatory Genes





RR1 RR2 RR3	RR4	sRR1 RR5	ctrA	flbD cheY cheYII cheYIII	sRR2 sRR3 sRR4 RR6 pleD	divK RR7 RR8	10 8	response regulators single domain response regulators
divJ HK1 HK2 HK3	HK4	cckA pleC HK5		HK-RR1 HK6	HK-RR2 cheAI cheAII	HK-RR3 HK-RR4 HK7	11 5	histidine kinases histidine kinases fused to response regulator domains
	sigT sigU			rpoD rpoN		sigC	5	sigma factors

Key regulatory proteins are both spatially and temporally regulated . . .



Key questions

- Mechanism for localization
- Structures at the membrane
- Reactions and reaction kinetics